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Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples.

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We compared and statistically evaluated the effectiveness of nine DNA extraction procedures by using frozen and dried samples of two silt loam soils and a silt loam wetland sediment with different organic matter contents. The effects of different chemical extractants (sodium dodecyl sulfate [SDS], chloroform, phenol, Chelex 100, and guanadinium isothiocyanate), different physical disruption methods (bead mill homogenization and freeze-thaw lysis), and lysozyme digestion were evaluate based on the yield and molecular size of the recovered DNA. Pairwise comparison of the nine extraction procedures revealed that bead mill homogenization with SD combined with either chloroform or phenol optimized both the amount of DNA extracted and the molecular size of the DNA (maximum size, 16 to 20 kb). Neithe lysozyme digestion before SDS treatment nor guanidine isothiocyanate treatment n addition of Chelex 100 resin improved the DNA yields. Bead mill homogenization a lysis mixture containing chloroform, SDS, NaCl, and phosphate-Tris buffer (pH was found to be the best physical lysis technique when DNA yield and cell lysis efficiency were used as criteria. The bead mill homogenization conditions were als optimized for speed and duration with two different homogenizers. Recovery of high-molecular-weight DNA was greatest when we used lower speeds and shorter times (30 to 120 s). We evaluated four different DNA purification methods (silica-based DNA binding, agarose gel electrophoresis, ammonium acetate precipitation, and Sephadex G-200 gel filtration) for DNA recovery and removal o PCR inhibitors from crude extracts. Sephadex G-200 spin column purification was found to be the best method for removing PCR-inhibiting substances while minimizing DNA loss during purification. Our results indicate that for these types samples, optimum DNA recovery requires brief, low-speed bead mill homogenization in the presence of a phosphate-buffered SDS-chloroform mixture, followed by Sephadex G-200 column purification.

PMID: 10543776 [PubMed - indexed for MEDLINE]